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(54) **Synthetic antigens for the detection of antibodies to hepatitis C virus.**

(57) Peptide sequences having the amino acid sequences given in the Sequence Listing (Sequence ID No. 1 to 20) are provided which are capable of mimicking proteins encoded by HCV for use as reagents for screening of blood and blood products for prior exposure to HCV. The peptides are at least 5 amino acids long and can be used in various specific assays for the detection of antibodies to HCV, for the detection of HCV antigens, or as immunogens.

EP 0 489 968 A1

The implementation of systematic testing for hepatitis B virus (HBV) has been instrumental in eliminating this virus from the blood supply. Nevertheless, a significant number of post-transfusion hepatitis (PTH) cases still occur. These cases are generally attributable to non-A, non-B hepatitis (NANBH) virus(es), the diagnosis of which is usually made by exclusion of other viral markers.

The etiological agent responsible for a large proportion of these cases has recently been cloned (Choo, Q-L et al. Science (1988) 244:359-362) and a first-generation antibody test developed (Kuo, G. et al. Science (1989) 244:362-364). The agent has been identified as a positive-stranded RNA virus, and the sequence of its genome has been partially determined. Studies suggest that this virus, referred to subsequently as hepatitis C virus (HCV), may be related to flaviviruses and pestiviruses. A portion of the genome of an HCV isolated from a chimpanzee ($HCV_{CDC/CHI}$) is disclosed in EPO 88310922.5. The coding sequences disclosed in this document do not include sequences originating from the 5'-end of the viral genome which code for putative structural proteins. Recently however, sequences derived from this region of the HCV genome have been published (Okamoto, H. et al., Japan J. Exp. Med. 60:167-177, 1990.). The amino acid sequences encoded by the Japanese clone HC-J1 were combined with the $HCV_{CDC/CHI}$ sequences in a region where the two sequences overlap to generate the composite sequence depicted in Figure 1. Specifically, the two sequences were joined at $glycine_{451}$. It should be emphasized that the numbering system used for the HCV amino acid sequence is not intended to be absolute since the existence of variant HCV strains harboring deletions or insertions is highly probable. Sequences corresponding to the 5' end of the HCV genome have also recently been disclosed in EPO 90302866.0.

In order to detect potential carriers of HCV, it is necessary to have access to large amounts of viral proteins. In the case of HCV, there is currently no known method for culturing the virus, which precludes the use of virus-infected cultures as a source of viral antigens. The current first-generation antibody test makes use of a fusion protein containing a sequence of 363 amino acids encoded by the HCV genome. It was found that antibodies to this protein could be detected in 75 to 85% of chronic NANBH patients. In contrast, only approximately 15% of those patients who were in the acute phase of the disease, had antibodies which recognized this fusion protein (Kuo, G. et al. Science (1989) 244:362-364). The absence of suitable confirmatory tests, however, makes it difficult to verify these statistics. The seeming similarity between the HCV genome and that of flaviviruses makes it possible to predict the location of epitopes which are likely to be of diagnostic value. An analysis of the HCV genome reveals the presence of a continuous long open reading frame. Viral RNA is presumably translated into a long polyprotein which is subsequently cleaved by cellular and/or viral proteases. By analogy with, for example, Dengue virus, the viral structural proteins are presumed to be derived from the amino-terminal third of the viral polyprotein. At the present time, the precise sites at which the polyprotein is cleaved can only be surmised. Nevertheless, the structural proteins are likely to contain epitopes which would be useful for diagnostic purposes, both for the detection of antibodies as well as for raising antibodies which could subsequently be used for the detection of viral antigens. Similarly, domains of nonstructural proteins are also expected to contain epitopes of diagnostic value, even though these proteins are not found as structural components of virus particles.

Brief Description of the Drawings

Figure 1 shows the amino acid sequence of the composite $HCV_{HC-J1/CDC/CHI}$
Figure 2 shows the antibody binding to individual peptides and various mixtures in an ELISA assay

Description of the Specific Embodiments

It is known that RNA viruses frequently exhibit a high rate of spontaneous mutation and, as such, it is to be expected that no two HCV isolates will be completely identical, even when derived from the same individual. For the purpose of this disclosure, a virus is considered to be the same or equivalent to HCV if it exhibits a global homology of 60 percent or more with the $HCV_{HC-J1/CDC/CHI}$ composite sequence at the nucleic acid level and 70 percent at the amino acid level.

Peptides are described which immunologically mimic proteins encoded by HCV. In order to accommodate strain-to-strain variations in sequence, conservative as well as non-conservative amino acid substitutions may be made. These will generally account for less than 35 percent of a specific sequence. It may be desirable in cases where a peptide corresponds to a region in the HCV polypeptide which is highly polymorphic, to vary one or more of the amino acids so as to better mimic the different epitopes of different viral strains.

The peptides of interest will include at least five, sometimes six, sometimes eight, sometimes twelve, usually fewer than about fifty, more usually fewer than about thirty-five, and preferably fewer than about

twenty-five amino acids included within the sequence encoded by the HCV genome. In each instance, the peptide will preferably be as small as possible while still maintaining substantially all of the sensitivity of the larger peptide. It may also be desirable in certain instances to join two or more peptides together in one peptide structure.

It should be understood that the peptides described need not be identical to any particular HCV sequence, so long as the subject compounds are capable of providing for immunological competition with at least one strain of HCV. The peptides may therefore be subject to insertions, deletions, and conservative or non-conservative amino acid substitutions where such changes might provide for certain advantages in their use.

Substitutions which are considered conservative are those in which the chemical nature of the substitute is similar to that of the original amino acid. Combinations of amino acids which could be considered conservative are Gly, Ala; Asp, Glu; Asn, Gln; Val, Ile, Leu; Ser, Thr; Lys, Arg; and Phe, Tyr.

Furthermore, additional amino acids or chemical groups may be added to the amino- or carboxyl terminus for the purpose of creating a "linker arm" by which the peptide can conveniently be attached to a carrier. The linker arm will be at least one amino acid and may be as many as 60 amino acids but will most frequently be 1 to 10 amino acids. The nature of the attachment to a solid phase or carrier need not be covalent.

Natural amino acids such as cysteine, lysine, tyrosine, glutamic acid, or aspartic acid may be added to either the amino- or carboxyl terminus to provide functional groups for coupling to a solid phase or a carrier. However, other chemical groups such as, for example, biotin and thioglycolic acid, may be added to the termini which will endow the peptides with desired chemical or physical properties. The termini of the peptides may also be modified, for example, by N-terminal acetylation or terminal carboxy-amidation. The peptides of interest are described in relation to the composite amino acid sequence shown in Figure 1. The amino acid sequences are given in the conventional and universally accepted three-letter code. In addition to the amino acids shown, other groups are defined as follows: Y is, for example, NH_2 , one or more N-terminal amino acids, or other moieties added to facilitate coupling. Y may itself be modified by, for example, acetylation. Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking. X is intended to represent OH, NH_2 , or a linkage involving either of these two groups.

Peptide I corresponds to amino acids 1 to 20 and has the following amino acid sequences:

(I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X.

Peptide II corresponds to amino acids 7 to 26 and has the amino acid sequence:

(II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X.

Of particular interest is the oligopeptide IIA:

(IIA) Y-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Z-X.

Peptide III corresponds to amino acids 13 to 32 and has the sequence:

(III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Z-X.

Peptide IV corresponds to amino acid 37 to 56 and has the sequences:

(IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X.

Peptide V corresponds to amino acids 49 to 68 and has the sequence:

(V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X.

Peptide VI corresponds to amino acid 61 to 80 and has the following sequence:

(VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X.

Peptide VII corresponds to amino acids 73 to 92 and has the sequence:

(VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Z-X.

Peptide VIII corresponds to amino acids 1688 to 1707 and has the sequence:

(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X.

Peptide IX corresponds to amino acids 1694 to 1713 and has the sequence:

(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X.

Peptide X corresponds to amino acids 1706 to 1725 and has the sequence:

(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X.

Peptide XI corresponds to amino acids 1712 to 1731 and has the sequence:

(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X.

Peptide XII corresponds to amino acids 1718 to 1737 and has the sequence:

(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X.

Peptide XIII corresponds to amino acids 1724 to 1743 and has the sequence:

(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X.

Peptide XIV corresponds to amino acids 1730 to 1749 and has the sequence:

(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X.

Peptide XV corresponds to amino acids 2263 to 2282 and has the sequence:

(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Z-X.

Peptide XVI corresponds to amino acids 2275 to 2294 and has the sequence:

(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Z-X.

Peptide XVII corresponds to amino acids 2287 to 2306 and has the sequence:

(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X.

Peptide XVIII corresponds to amino acids 2299 to 2318 and has the sequence:

(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X.

Peptide XIX corresponds to amino acids 2311 to 2330 and has the sequence:

(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X.

Of particular interest is the use of the mercapto-group of cysteines or thioglycolic acids used for acylating terminal amino groups for cyclizing the peptides or coupling two peptides together. The cyclization or coupling may occur via a single bond or may be accomplished using thiol-specific reagents to form a molecular bridge.

The peptides may be coupled to a soluble carrier for the purpose of either raising antibodies or facilitating the adsorption of the peptides to a solid phase. The nature of the carrier should be such that it has a molecular weight greater than 5000 and should not be recognized by antibodies in human serum. Generally, the carrier will be a protein. Proteins which are frequently used as carriers are keyhole limpet hemocyanin, bovine gamma globulin, bovine serum albumin, and poly-L-lysine.

There are many well described techniques for coupling peptides to carriers. The linkage may occur at the N-terminus, C-terminus or at an internal site in the peptide. The peptide may also be derivatized for coupling. Detailed descriptions of a wide variety of coupling procedures are given, for example, in Van Regenmortel, M.H.V., Briand, J.P., Muller, S., and Plaué, S., Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 19, Synthetic Polypeptides as Antigens, Elsevier Press, Amsterdam, New York, Oxford, 1988.

The peptides may also be synthesized directly on an oligo-lysine core in which both the alpha as well as the epsilon-amino groups of lysines are used as growth points for the peptides. The number of lysines comprising the core is preferably 3 or 7. Additionally, a cysteine may be included near or at the C-terminus of the complex to facilitate the formation of homo- or heterodimers. The use of this technique has been amply illustrated for hepatitis B antigens (Tam, J.P., and Lu, Y-A., Proc. Natl. Acad. Sci. USA (1989) 86:9084-9088) as well as for a variety of other antigens (see Tam, J.P., Multiple Antigen Peptide System: A Novel Design for Synthetic Peptide Vaccine and Immunoassay, in Synthetic Peptides, Approaches to Biological Problems, Tam, J.P., and Kaiser, E.T., ed. Alan R. Liss Inc., New York, 1989).

Depending on their intended use, the peptides may be either labeled or unlabeled. Labels which may be employed may be of any type, such as enzymatic, chemical, fluorescent, luminescent, or radioactive. In addition, the peptides may be modified for binding to surfaces or solid phases, such as, for example, microtiter plates, nylon membranes, glass or plastic beads, and chromatographic supports such as cellulose, silica, or agarose. The methods by which peptides can be attached or bound to solid support or surface are well known to those versed in the art.

Of particular interest is the use of mixtures of peptides for the detection of antibodies specific for hepatitis C virus. Mixtures of peptides which are considered particularly advantageous are:

A. II, III, V, IX, and XVIII

B. I, II, V, IX, XI, XVI, and XVIII

C. II, III, IV, V, VIII, XI, XVI, and XVIII

D. II, IX, and XVIII

E. II, III, IV, and V

F. VIII, IX, XI, XIII, and XIV

G. XV, XVI, XVII, XVIII, and XIX

Antibodies which recognize the peptides can be detected in a variety of ways. A preferred method of detection is the enzyme-linked immunosorbant assay (ELISA) in which a peptide or mixture of peptides is bound to a solid support. In most cases, this will be a microtiter plate but may in principle be any sort of insoluble solid phase. A suitable dilution or dilutions of serum or other body fluid to be tested is brought into contact with the solid phase to which the peptide is bound. The incubation is carried out for a time necessary to allow the binding reaction to occur. Subsequently, unbound components are removed by washing the solid phase. The detection of immune complexes is achieved using antibodies which specifically bind to human immunoglobulins, and which have been labeled with an enzyme, preferably but not limited to either horseradish peroxidase, alkaline phosphatase, or beta-galactosidase, which is capable of converting a colorless or nearly colorless substrate or co-substrate into a highly colored product or a

product capable of forming a colored complex with a chromogen. Alternatively, the detection system may employ an enzyme which, in the presence of the proper substrate(s), emits light. The amount of product formed is detected either visually, spectrophotometrically, electrochemically, or luminometrically, and is compared to a similarly treated control. The detection system may also employ radioactively labeled antibodies, in which case the amount of immune complex is quantified by scintillation counting or gamma counting.

Other detection systems which may be used include those based on the use of protein A derived from Staphylococcus aureus Cowan strain I, protein G from group C Staphylococcus sp. (strain 26RP66), or systems which make use of the high affinity biotin-avidin or streptavidin binding reaction.

Antibodies raised to carrier-bound peptides can also be used in conjunction with labeled peptides for the detection of antibodies present in serum or other body fluids by competition assay. In this case, antibodies raised to carrier-bound peptides are attached to a solid support which may be, for example, a plastic bead or a plastic tube. Labeled peptide is then mixed with suitable dilutions of the fluid to be tested and this mixture is subsequently brought into contact with the antibody bound to the solid support. After a suitable incubation period, the solid support is washed and the amount of labeled peptide is quantified. A reduction in the amount of label bound to the solid support is indicative of the presence of antibodies in the original sample. By the same token, the peptide may also be bound to the solid support. Labeled antibody may then be allowed to compete with antibody present in the sample under conditions in which the amount of peptide is limiting. As in the previous example, a reduction in the measured signal is indicative of the presence of antibodies in the sample tested.

Another preferred method of antibody detection is the homogeneous immunoassay. There are many possible variations in the design of such assays. By way of example, numerous possible configurations for homogeneous enzyme immunoassays and methods by which they may be performed are given in Tijssen, P., Practice and Theory of Enzyme Immunoassays, Elsevier Press, Amersham, Oxford, New York, 1985. Detection systems which may be employed include those based on enzyme channeling, bioluminescence, allosteric activation and allosteric inhibition. Methods employing liposome-entrapped enzymes or coenzymes may also be used (see Pinnaduwage, P. and Huang, L., Clin. Chem. (1988) 34/2: 268-272, and Ullman, E.F. et al., Clin. Chem. (1987) 33/9: 1579-1584 for examples).

The synthesis of the peptides can be achieved in solution or on a solid support. Synthesis protocols generally employ the use t-butyloxycarbonyl- or 9-fluorenylmethoxy-carbonyl-protected activated amino acids. The procedures for carrying out the syntheses, the types of side-chain protection, and the cleavage methods are amply described in, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2nd Edition, Pierce Chemical Company, 1984; and Atherton and Sheppard, Solid Phase Peptide Synthesis, IRL Press, 1989.

Experimental

I. Peptide Synthesis

All of the peptides described were synthesized on Pepsyn K polyamide-Kieselguhr resin (Milligen, Novato, California) which had been functionalized with ethylenediamine and onto which the acid-labile linker 4-(alpha-Fmoc-amino-2',4'-dimethoxybenzyl) phenoxyacetic acid had been coupled (Rink, Tetrahedron Lett. (1987) 28:3787). t-Butyl-based side-chain protection and Fmoc alpha-amino-protection was used. The guanidino-group of arginine was protected by the 2,2,5,7,8-pentamethylchroman-6-sulfonyl moiety. The imidazole group of histidine was protected by either t-Boc or trityl and the sulfhydryl group of cysteine was protected by a trityl group. Couplings were carried out using performed O-pentafluorophenyl esters except in the case of arginine where diisopropylcarbodiimide-mediated hydroxybenzotriazole ester formation was employed. Except for peptide I, all peptides were N-acetylated using acetic anhydride. All syntheses were carried out on a Milligen 9050 PepSynthesizer (Novato, California) using continuous flow procedures. Following cleavage with trifluoroacetic acid in the presence of scavengers and extraction with diethylether, all peptides were analyzed by C_{18} -reverse phase chromatography.

II. Detection of Antibodies to Hepatitis C Virus

A. Use of peptides bound to a nylon membrane.

Peptides were dissolved in a suitable buffer to make a concentrated stock solution which was then further diluted in phosphate-buffered saline (PBS) or sodium carbonate buffer, pH 9.6 to make working

solutions. The peptides were applied as lines on a nylon membrane (Pall, Portsmouth, United Kingdom), after which the membrane was treated with casein to block unoccupied binding sites. The membrane was subsequently cut into strips perpendicular to the direction of the peptide lines. Each strip was then incubated with a serum sample diluted 1 to 100, obtained from an HCV-infected individual. Antibody binding was detected by incubating the strips with goat anti-human immunoglobulin antibodies conjugated to the enzyme alkaline phosphatase. After removing unbound conjugate by washing, a substrate solution containing 5-bromo-4-chloro-3-indolylphosphate and nitro blue tetrazolium was added.

Positive reactions are visible as colored lines corresponding to the positions of the peptides which are specifically recognized. The reaction patterns of thirty-six different sera are tabulated in Table 1. The results shown in Table 1 are further summarized in Table 2.

B. Use of peptides in an enzyme-linked immunosorbent assay (ELISA).

Peptide stock solutions were diluted in sodium carbonate buffer, pH 9.6 and used to coat microtiter plates at a peptide concentration of 2 micrograms per milliliter. A mixture consisting of peptides II, III, V, IX, and XVIII was also used to coat plates. Following coating, the plates were blocked with casein. Fifteen HCV-antibody-positive sera and control sera from seven uninfected blood donors were diluted 1 to 20 and incubated in wells of the peptide-coated plates. Antibody binding was detected by incubating the plates with goat anti-human immunoglobulin antibodies conjugated to the enzyme horseradish peroxidase. Following removal of unbound conjugate by washing, a solution containing H_2O_2 and 3,3',5,5'-tetramethylbenzidine was added. Reactions were stopped after a suitable interval by addition of sulfuric acid. Positive reactions gave rise to a yellow color which was quantified using a conventional microtiter plate reader. The results of these determinations are tabulated in Table 3. To correct for any aspecific binding which could be attributable to the physical or chemical properties of the peptides themselves, a cut-off value was determined for each peptide individually. This cut-off absorbance value was calculated as the average optical density of the negative samples plus 0.200. Samples giving absorbance values higher than the cut-off values are considered positive. The results for the fifteen positive serum samples are further summarized in Table 4.

While it is evident that some of the peptides are recognized by a large percentage of sera from HCV-infected individuals, it is also clear that no single peptide is recognized by all sera. In contrast, the peptide mixture was recognized by all fifteen sera and, for six of the fifteen sera, the optical densities obtained were equal to or higher than those obtained for any of the peptides individually. These results serve to illustrate the advantages of using mixtures of peptides for the detection of anti-HCV antibodies.

C. Binding of antibodies in sera from HCV-infected patients to various individual peptides and peptide mixtures in an ELISA.

Five peptides were used individually and in seven different combinations to coat microtiter plates. The plates were subsequently incubated with dilutions of fifteen HCV antibody-positive sera in order to evaluate the relative merits of using mixtures as compared to individual peptides for antibody detection. The mixtures used and the results obtained are shown in Figure 2.

In general, the mixtures functioned better than individual peptides. This was particularly evident for mixture 12 (peptides I, III, V, IX, and XVIII) which was recognized by all twelve of the sera tested. These results underscore the advantages of using mixtures of peptides in diagnostic tests for the detection of antibodies to HCV.

D. Use of a mixture of peptides in an ELISA assay for the detection of anti-HCV antibodies.

A mixture of peptides II, III, V, IX, and XVIII was prepared and used to coat microtiter plates according to the same procedure used to test the individual peptides. A total of forty-nine sera were tested from patients with clinically diagnosed but undifferentiated chronic non A non B hepatitis as well as forty-nine sera from healthy blood donors. Detection of antibody binding was accomplished using goat anti-human immunoglobulin antibodies conjugated to horseradish peroxidase. The resulting optical density values are given in Table 5. These results indicate that the mixture of peptides is not recognized by antibodies in sera from healthy donors (0/49 reactives) but is recognized by a large proportion (41/49, or 84%) of the sera from patients with chronic NANBH. These results demonstrate that the peptides described can be used effectively as mixtures for the diagnosis of HCV infection.

E. Detection of anti-HCV antibodies in sera from patients with acute NANB infection using individual peptides bound to nylon membranes and a mixture of peptides in an ELISA assay, and comparison with a commercially available kit.

Peptides were applied to nylon membranes or mixed and used to coat microtiter plates as previously described. The peptide mixture consisted of peptides II, III, V, IX, and XVIII. Sera obtained from twenty-nine patients with acute non-A, non-B hepatitis were then tested for the presence of antibodies to hepatitis C virus. These same sera were also evaluated using a commercially available kit (Ortho, Emeryville, CA, USA).

The results of this comparative study are given in Table 6. In order to be able to compare the peptide-based ELISA with the commercially available kit, the results for both tests are also expressed as signal to noise ratios (S/N) which were calculated by dividing the measured optical density obtained for each sample by the cut-off value. A signal-to-noise ratio greater or equal to 1.0 is taken to represent a positive reaction. For the commercially available kit, the cut-off value was calculated according to the manufacturer's instructions. The cut-off value for the peptide-based ELISA was calculated as the average optical density of five negative samples plus 0.200.

The scale used to evaluate antibody recognition of nylon-bound peptides was the same as that given in Table 1. Of the twenty-nine samples tested, twenty-five (86%) were positive in the peptide-based ELISA and recognized one or more nylon-bound peptides. In contrast, only fourteen of the twenty-nine sera scored positive in the commercially available ELISA. These results serve to illustrate the advantages of using peptide mixtures for the detection of anti-HIV antibodies as well as the need to include in the mixtures peptides which contain amino acid sequences derived from different regions of the HCV polyprotein.

Peptide

Serum nr.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
1			3	1				1			0.5					2	2	1	1
2											0.5		1	2		2	1		
3	1	0.5	2	1		0.5		2	0.5	1	0.5								
4													1						
6			2	1	0.5											2			
7	0.5	1	2	1	0.5			3	2	2	1		1		2		0.5	1	1
8	0.5	1	3	1	1		1	1		2	1		1			1	1	0.5	
10		1	0.5					3	1	1				0.5	2	2		2	2
13	0.5	0.5	2		0.5					1			1	0.5		0.5			
15				0.5				2	1	0.5				1		0.5			
16	2	1	0.5	0.5	1	0.5		2	0.5	1					2	2	1	2	
18	1	1	3	0.5		2	0.5									1	0.5		
23	0.5		1	1			0.5			1	0.5		0.5		0.5				
24	1	0.5	2	1	0.5	0.5	0.5	2								1			
25			1	0.5				2	0.5	0.5	2		1	1		2			
26								1								0.5			
27	0.5	0.5	1					3	2							1	2	1	0.5
29		0.5	3	2	1	1	0.5			2	1		1	1	2	2	1	1	1
30		0.5	0.5	1	1	0.5													
31		1	0.5							0.5	0.5				0.5				
32		1	2							1	0.5				1	1			
33								0.5		1	0.5							0.5	
34		1	1	1				3	1							1	1	0.5	
35	1	1	2	1	1	1	0.5												
36	1		2	1		1													
37	1	1																	
44		1	2	1	0.5										2		0.5		
46		0.5	2	0.5	0.5		0.5	2											
47		0.5		0.5		0.5		1										1	1
48	1	2		2			0.5	2					0.5		1	1		1	0.5
49		1	1	0.5	0.5	0.5				0.5	0.5		0.5				1		
50		1	2	1				2	0.5	1	1		1			1	1	0.5	0.5
51			2	0.5	0.5		0.5									1	1	0.5	
52			2	0.5			0.5			0.5									
54			2		0.5	0.5	1	0.5		1	1		1			1	1	1	
56	ND	ND	ND	ND	ND	ND	ND	2			0.5	1	2	1					

Table 1. Recognition of peptides bound to nylon membranes by sera from persons infected by HCV.

Blank: no reaction; 0.5: weakly positive; 1: clearly positive; 2: strong reaction; 3: intense reaction; ND: not determined

Table 2.

Summary of antibody binding to nylon-bound HCV peptides by sera from infected patients.		
Peptide	No. reactive sera	% reactive sera
I	13/35	37
II	22/35	63
III	27/35	77
IV	24/35	69
V	14/35	40
VI	11/35	31
VII	11/35	31
VIII	19/36	53
IX	9/36	25
X	17/36	47
XI	15/36	42
XII	1/36	3
XIII	13/36	36
XIV	7/36	19
XV	9/36	25
XVI	20/36	56
XVII	14/36	39
XVIII	14/36	39
XIX	8/36	22

Table 3. Comparison of Individual Peptides in an ELISA Assay for the Detection of Antibodies to HCV.

sample ident	peptide																		
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
1	0.786	1.119	1.284	0.265	0.042	0.04	0.05	0.571	0.659	0.048	0.04	0.043	0.068	0.044	0.041	1.063	0.956	1.383	1.348
2	0.044	0.039	0.11	0.041	0.037	0.038	0.039	0.479	0.78	0.169	0.563	0.039	0.042	0.515	0.039	0.64	0.319	0.154	0.49
3	0.815	0.944	0.825	0.399	0.654	0.487	0.32	0.705	0.965	0.468	0.668	0.041	0.093	0.341	0.043	0.292	0.038	0.046	0.038
7	1.122	1.23	0.588	0.682	0.659	0.182	0.107	0.907	1.42	0.663	0.646	0.041	0.235	0.068	0.575	0.042	0.041	0.872	1.271
8	1.155	1.159	1.2	0.508	1.272	0.433	0.623	0.61	0.863	0.752	1.175	0.046	0.42	0.102	0.068	0.552	0.671	0.417	0.058
10	1.009	1.236	1.083	0.044	0.508	0.042	0.073	1.49	1.529	0.689	0.834	0.041	0.044	0.314	0.793	0.886	0.037	1.335	1.356
11	0.048	0.051	0.478	0.052	0.119	0.039	0.1	0.634	0.711	0.199	0.967	0.125	0.454	0.088	0.111	0.274	0.093	0.838	0.065
15	0.224	0.602	0.813	0.093	0.068	0.077	0.147	0.807	1.225	0.315	0.688	0.046	0.154	0.202	0.065	0.372	0.097	0.155	0.077
23	0.62	0.8	0.924	0.568	0.759	0.442	0.683	0.089	0.121	0.422	0.898	0.041	0.049	0.101	0.068	0.311	0.038	0.052	0.05
24	1.042	1.132	1.026	0.518	0.916	0.302	0.253	1.013	1.364	0.236	0.397	0.054	0.123	0.078	0.051	0.416	0.053	0.1	0.085
49	0.624	0.73	0.884	0.171	0.372	0.055	0.04	0.084	0.064	0.209	0.731	0.044	0.113	0.039	0.044	0.299	0.038	0.192	0.041
13	0.76	0.857	0.815	0.087	0.422	0.098	0.045	0.473	0.469	0.529	0.735	0.043	0.044	0.186	0.043	0.088	0.037	0.066	0.04
31	0.84	1.114	0.445	0.672	0.046	0.041	0.042	0.184	0.15	0.255	0.69	0.041	0.04	0.061	0.136	0.292	0.038	0.224	0.501
47	1.303	1.53	1.238	0.751	0.83	0.629	0.073	0.545	0.739	0.044	0.041	0.041	0.041	0.498	0.04	0.268	0.042	1.288	1.206
56	1.169	1.301	1.364	1.269	1.374	0.85	1.066	1.45	1.523	0.079	1.069	0.058	0.588	0.038	0.039	0.218	0.038	0.087	0.039
bd A28	0.054	0.043	0.139	0.045	0.135	0.042	0.041	0.088	0.115	0.044	0.042	0.044	0.052	0.043	0.043	0.307	0.042	0.045	0.061
bd A169	0.041	0.042	0.134	0.044	0.038	0.04	0.041	0.061	0.07	0.043	0.042	0.041	0.04	0.041	0.041	0.255	0.038	0.058	0.042
bd A170	0.04	0.044	0.117	0.04	0.038	0.04	0.04	0.081	0.05	0.04	0.039	0.04	0.038	0.038	0.144	0.292	0.038	0.058	0.039
bd A171	0.041	0.048	0.148	0.043	0.037	0.045	0.046	0.077	0.065	0.043	0.041	0.043	0.039	0.04	0.045	0.288	0.037	0.05	0.04
bd A166	0.047	0.048	0.124	0.044	0.038	0.042	0.041	0.056	0.066	0.041	0.041	0.042	0.04	0.041	0.041	0.207	0.039	0.046	0.041
bd A165	0.041	0.048	0.123	0.043	0.035	0.051	0.042	0.051	0.091	0.041	0.04	0.042	0.039	0.043	0.039	0.253	0.034	0.08	0.098
AVG	0.044	0.045	0.131	0.043	0.053	0.043	0.042	0.069	0.078	0.042	0.041	0.042	0.041	0.041	0.059	0.267	0.038	0.053	0.054
STD	0.005	0.002	0.011	0.002	0.037	0.004	0.002	0.013	0.021	0.001	0.001	0.001	0.005	0.002	0.038	0.033	0.002	0.006	0.021
cut off	0.109	0.101	0.214	0.099	0.214	0.105	0.098	0.158	0.189	0.095	0.094	0.095	0.106	0.097	0.223	0.416	0.004	0.121	0.167

Table 4

Summary of antibody-binding to individual peptides in an ELISA assay.		
Peptide	No. reactive sera	% reactive sera
I	13	87
II	13	87
III	14	93
IV	10	67
V	10	67
VI	7	47
VII	8	53
VIII	13	87
IX	12	80
X	13	87
XI	13	87
XII	1	7
XIII	7	47
XIV	8	53
XV	2	13
XVI	5	33
XVII	4	27
XVIII	10	67
XIX	6	40

Table 5

Use of a peptide mixture for the detection of antibodies to HCV in sera from chronic NANBH patients and comparison to sera from healthy blood donors.			
Chronic NANB Sera		Control Sera	
Serum nr.	Optical Density	Serum nr.	Optical Density
101	0.041	1	0.049
102	1.387	2	0.047
103	1.578	3	0.049
104	1.804	4	0.046
105	1.393	5	0.049
107	1.604	6	0.045
108	1.148	7	0.043
109	1.714	8	0.053
110	1.692	9	0.049
112	0.919	10	0.047
113	1.454	11	0.060
114	0.936	12	0.044
115	0.041	13	0.049
116	1.636	14	0.051
118	1.242	15	0.056
119	1.568	16	0.050
120	1.290	17	0.049
121	1.541	18	0.055
122	1.422	19	0.054
123	1.493	20	0.058
124	1.666	21	0.050
125	1.644	22	0.044
126	1.409	23	0.043
127	1.625	24	0.045
128	1.061	25	0.046
129	1.553	26	0.049
130	1.709	27	0.050
131	0.041	28	0.047
132	0.044	29	0.050
133	1.648	30	0.053
134	0.043	31	0.051
135	1.268	32	0.053
136	1.480	33	0.055
138	0.628	34	0.064
139	0.042	35	0.063
140	0.040	36	0.057
141	0.039	38	0.048
142	1.659	39	0.045
143	1.457	40	0.046
144	0.722	41	0.046
145	1.256	42	0.051
146	0.373	43	0.057
147	1.732	44	0.050
148	1.089	45	0.050
149	1.606	46	0.045
150	1.725	47	0.041
151	1.449	48	0.064
154	1.639	49	0.040
155	1.775	50	0.036

Table 6. Comparison of anti-HCV antibody detection by nylon-bound peptides, a peptide-based ELISA, and a commercially available kit.

Serum nr.	Nylon-bound peptides I	III	IV	V	VI	VIII	XI	XIV	XV	XVI	XVII	Optical density Peptide ELISA	S/N	Optical density Commercial ELISA	S/N
191	0	0	0	0	0	0	0	0	0	0	0	0.045	0.18	0.295	0.47
192	0	0	0	0	0	0	0	0	0	0	0	0.042	0.17	0.289	0.46
193	0	0	0	0	0	0	0	0	0	0	0	0.039	0.16	0.197	0.32
194	0	0	0	0	0	0	0	0	0	0	0	0.044	0.18	0.183	0.29
195	1	2	2	3	0	0	0.5	0.5	1	3	1	1.692	6.77	3.000*	4.82*
196	1	2	1	2	0.5	0.5	0.5	0.5	0.5	2	0	1.569	6.28	0.386	0.62
197	1	2	1	2	0	0.5	0.5	0.5	1	2	0	1.523	6.09	0.447	0.72
198	1	2	2	2	0	0	0	0	1	2	0	1.578	6.31	0.354	0.57
211	0.5	1	0.5	0.5	0	2	2	0	2	0	1	1.606	6.42	3.000*	4.82*
213	0	0	0	1	0	0	0	0	0	0	0	0.369	1.48	0.127	0.20
214	0	0	0	1	0	0	0	0	0	0	0	0.444	1.78	0.101	0.16
215	0	0	0	1	0	0	0	0	0	0	0	0.637	2.55	0.101	0.16
216	0	0	0	0.5	0	0	0	0	0	0	0	0.812	3.25	0.092	0.15
217	0	0	0	1	0	0	0	0	0	0	0	1.320	5.28	0.875	1.40
219	0.5	1	1	2	1	0.5	1	0	0.5	0.5	1	1.547	6.19	3.000*	4.82*
220	0.5	1	1	2	1	0.5	1	0	0.5	0.5	1	1.536	6.14	3.000*	4.82*
221	0	0	0	0.5	0	0	0	0	0	0	0	1.428	5.71	0.327	0.52
222	1	1	1	1	0	0	2	0.5	0.5	0	0	1.362	5.45	3.000*	4.82*
223	1	1	1	1	0	0	3	0.5	0.5	0	0	1.316	5.26	3.000*	4.82*
224	1	1	2	1	0	0.5	3	0.5	0.5	0	0	1.304	5.22	3.000*	4.82*
225	0	0	0	0	0	0.5	0.5	0.5	0	0	2	1.178	4.71	2.398	3.85
226	0.5	0	0	0	0	2	3	2	0.5	0.5	3	1.286	5.14	3.000*	4.82*
227	0	0	0	0	0	2	2	0.5	0.5	0.5	2	1.335	5.34	3.000*	4.82*
228	0.5	0	0.5	0.5	0	2	2	2	0	0	2	1.400	5.60	3.000*	4.82*
234	0.5	0.5	0	0.5	0	0	3	1	3	1	3	1.481	5.92	3.000*	4.82*
235	0	0	0	0.5	0	0	0	0	0	0	0	0.351	1.40	0.257	0.41
236	0	0	0	0.5	0	0	0	0	0	0	0	0.475	1.90	0.245	0.39
237	0	0	0	1	0	0	0	0	0	0	0	1.134	4.54	0.351	0.56
238	0	0	0	1	0	1	1	0	0	0	0	1.096	4.38	1.074	1.72

Cut-off: 0.250 Cut-off: 0.623

0: no reaction; 0.5: weakly positive; 1: clearly positive; 2: strong reaction; 3: intense reaction;

* O.D. exceeded 3.000 and was out of range. The values given are therefore minimum values.

SEQUENCE LISTING

SEQ ID NO: 1
SEQUENCE TYPE: amino acid sequence

SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C Virus
FEATURES:	the peptide corresponds to amino acids 1 to 20 of the composite amino acid sequence shown in Figure 1.

Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn
1 5 10
Thr Asn Arg Arg Pro Gln
15 20

SEQ ID NO:	2
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 7 to 26 of the composite amino acid sequence shown in Figure 1.

Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10
Asp Val Lys Phe Pro Gly
15 20

SEQ ID NO:	3
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	11 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 8 to 18 of the composite amino acid sequence shown in Figure 1.

Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg
1 5 10

SEQ ID NO:	4
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 13 to 32 of the composite amino acid sequence shown in Figure 1.

Arg Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly
1 5 10
Gly Gly Gln Ile Val Gly
15 20

SEQ ID NO:	5
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids

MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 37 to 56 of the composite amino acid sequence shown in Figure 1.

```
Leu Pro Arg Arg  Gly Pro Arg Leu Gly    Val Arg Ala Thr Arg
1                5                      10
Lys Thr Ser Glu Arg    Ser
15                     20
```

SEQ ID NO:	6
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 49 to 68 of the composite amino acid sequence shown in Figure 1.

```
Thr Arg Lys Thr  Ser Glu Arg Ser Gln    Pro Arg Gly Arg Arg
1                5                      10
Gln Pro Ile Pro Lys    Val
15                     20
```

SEQ ID NO:	7
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 61 to 80 of the composite amino acid sequence shown in Figure 1.

```
Arg Arg Gln Pro  Ile Pro Lys Val Arg Arg Pro Glu Gly Arg
1                5                   10
Thr Trp Ala Gln Pro    Gly
15                     20
```

SEQ ID NO:	8
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 73 to 92 of the composite amino acid sequence shown in Figure 1.

```
Gly Arg Thr Trp  Ala Gln Pro Gly Tyr    Pro Trp Pro Leu Tyr
1                5                      10
Gly Asn Glu Gly Cys    Gly
15                     20
```

SEQ ID NO:	9
SEQUENCE TYPE:	amino acid sequence

SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 1688 to 1707 of the composite amino acid sequence shown in Figure 1.

Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu
1 5 10
Tyr Arg Glu Phe Asp Glu
15 20

SEQ ID NO:	10
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 1694 to 1713 of the composite amino acid sequence shown in Figure 1.

Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu
1 5 10
Met Glu Glu Cys Ser Gln
15 20

SEQ ID NO:	11
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 1706 to 1725 of the composite amino acid sequence shown in Figure 1.

Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro Tyr Ile Glu
1 5 10
Gln Gly Met Met Leu Ala
15 20

SEQ ID NO:	12
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids
MOLECULE TYPE:	peptide
ORIGINAL SOURCE ORGANISM:	hepatitis C virus
FEATURES:	the peptide corresponds to amino acids 1712 to 1731 of the composite amino acid sequence shown in Figure 1.

Ser Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala
1 5 10
Glu Gln Phe Lys Gln Lys
15 20

SEQ ID NO:	13
SEQUENCE TYPE:	amino acid sequence
SEQUENCE LENGTH:	20 amino acids

MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 1718 to 1737 of the composite amino acid sequence shown in Figure 1.

Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys
1 5 10
Ala Leu Gly Leu Leu Gln
15 20

SEQ ID NO: 14
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 1724 to 1743 of the composite amino acid sequence shown in Figure 1.

Leu Ala Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln
1 5 10
Thr Ala Ser Arg Gln Ala
15 20

SEQ ID NO: 15
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 1730 to 1749 of the composite amino acid sequence shown in Figure 1.

Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg Gln Ala
1 5 10
Glu Val Ile Ala Pro Ala
15 20

SEQ ID NO: 16
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 2263 to 2282 of the composite amino acid sequence shown in Figure 1.

Glu Asp Glu Arg Glu Ile Ser Val Pro Ala Glu Ile Leu Arg
1 5 10
Lys Ser Arg Arg Phe Ala
15 20

SEQ ID NO: 17
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids

MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 2275 to 2294 of the composite amino acid sequence shown in Figure 1.

Leu Arg Lys Ser Arg Arg Phe Ala Gln Ala Leu Pro Val Trp
1 5 10
Ala Arg Pro Asp Tyr Asn
15 20

SEQ ID NO: 18
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 2287 to 2306 of the composite amino acid sequence shown in Figure 1.

Val Trp Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Thr
1 5 10
Trp Lys Lys Pro Asp Tyr
15 20

SEQ ID NO: 19
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 2299 to 2318 of the composite amino acid sequence shown in Figure 1.

Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val Val His
1 5 10
Gly Cys Pro Leu Pro Pro
15 20

SEQ ID NO: 20
SEQUENCE TYPE: amino acid sequence
SEQUENCE LENGTH: 20 amino acids
MOLECULE TYPE: peptide
ORIGINAL SOURCE ORGANISM: hepatitis C virus
FEATURES: the peptide corresponds to amino acids 2311 to 2330 of the composite amino acid sequence shown in Figure 1.

Val His Gly Cys Pro Leu Pro Pro Pro Lys Ser Pro Pro Val
1 5 10
Pro Pro Pro Arg Lys Lys
15 20

Claims

1. A peptide of the formula:
(I) Y-Met-Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

2. A peptide of the formula:
(II) Y-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

3. A peptide of the formula:
(III) Y-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

4. A peptide of the formula:
(IV) Y-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

5. A peptide of the formula:
(V) Y-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

6. A peptide of the formula:
(VI) Y-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

7. A peptide of the formula:
(VII) Y-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

8. A peptide of the formula:
(VIII) Y-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

9. A peptide of the formula:
(IX) Y-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

10. A peptide of the formula:
(X) Y-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate

coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

11. A peptide of the formula:
(XI) Y-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

12. A peptide of the formula:
(XII) Y-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

13. A peptide of the formula:
(XIII) Y-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

14. A peptide of the formula:
(XIV) Y-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

15. A peptide of the formula:
(XV) Y-Glu-Asp-Glu-Arg-Glu-Ile-Ser-Val-Pro-Ala-Glu-Ile-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

16. A peptide of the formula:
(XVI) Y-Leu-Arg-Lys-Ser-Arg-Arg-Phe-Ala-Gln-Ala-Leu-Pro-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

17. A peptide of the formula:
(XVII) Y-Val-Trp-Ala-Arg-Pro-Asp-Tyr-Asn-Pro-Pro-Leu-Val-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

18. A peptide of the formula:
(XVIII) Y-Glu-Thr-Trp-Lys-Lys-Pro-Asp-Tyr-Glu-Pro-Pro-Val-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

19. A peptide of the formula:
(XIX) Y-Val-His-Gly-Cys-Pro-Leu-Pro-Pro-Pro-Lys-Ser-Pro-Pro-Val-Pro-Pro-Pro-Arg-Lys-Lys-Z-X.
Where Y is NH_2 , one or more N-terminal amino acids, or other chemical entities added to facilitate coupling; Z is a bond, (an) amino acid(s), or (a) chemical group(s) which may be used for linking; and X is OH, NH_2 , or a linkage involving either of these groups.

20. A composition comprising at least one of the peptides of claims 1 to 19.

21. A composition comprising at least one of the peptides of claims 1 to 19 attached to a carrier.

22. A method for the detection of antibodies to hepatitis C virus in a biological fluid such as serum or plasma, characterized by contacting body fluid of a person to be diagnosed with any of the peptides of claims 1 to 19 or compositions of claims 20 and 21, and detecting the immunological complex formed between said antibodies and the antigen(s) used.

23. The method of claim 22, characterized in that said detection of said immunological complex is achieved by reacting said immunological complex with a labeled reagent selected from anti-human immunoglobulin-antibodies or staphylococcal A protein or streptococcal G protein or avidin or streptavidin and detecting the complex formed reagent between said conjugate and said reagent.

24. A kit for the detection of anti-hepatitis C virus antibodies in a biological fluid, comprising:
   - a composition as defined in either of claims 20 or 21.
   - the means for detecting the immunological complex formed.

25. The kit of claim 24, characterized in that said means for detecting said immunological complex comprise anti-human immunoglobulin(s) or protein A or protein G or avidin or streptavidin and means for detecting the complex formed between the anti-HCV antibodies contained in the detected immunological conjugate.

Figure 1. Amino Acid Sequence of the Composite $HCV_{HC-J1/CDC/CH}$

					5					10					15
1	Met	Ser	Thr	Ile	Pro	Lys	Pro	Gln	Arg	Lys	Thr	Lys	Arg	Asn	Thr
16	Asn	Arg	Arg	Pro	Gln	Asp	Val	Lys	Phe	Pro	Gly	Gly	Gly	Gln	Ile
31	Val	Gly	Gly	Val	Tyr	Leu	Leu	Pro	Arg	Arg	Gly	Pro	Arg	Leu	Gly
46	Val	Arg	Ala	Thr	Arg	Lys	Thr	Ser	Glu	Arg	Ser	Gln	Pro	Arg	Gly
61	Arg	Arg	Gln	Pro	Ile	Pro	Lys	Val	Arg	Arg	Pro	Glu	Gly	Arg	Thr
76	Trp	Ala	Gln	Pro	Gly	Tyr	Pro	Trp	Pro	Leu	Tyr	Gly	Asn	Glu	Gly
91	Cys	Gly	Trp	Ala	Gly	Trp	Leu	Leu	Ser	Pro	Arg	Gly	Ser	Arg	Pro
106	Ser	Trp	Gly	Pro	Thr	Asp	Pro	Arg	Arg	Arg	Ser	Arg	Asn	Leu	Gly
121	Lys	Val	Ile	Asp	Thr	Leu	Thr	Cys	Gly	Phe	Ala	Asp	Leu	Met	Gly
136	Tyr	Ile	Pro	Leu	Val	Gly	Ala	Pro	Leu	Gly	Gly	Ala	Ala	Arg	Ala
151	Leu	Ala	His	Gly	Val	Arg	Val	Leu	Glu	Asp	Gly	Val	Asn	Tyr	Ala
166	Thr	Gly	Asn	Leu	Pro	Gly	Cys	Ser	Phe	Ser	Ile	Phe	Leu	Leu	Ala
181	Leu	Leu	Ser	Cys	Leu	Thr	Val	Pro	Ala	Ser	Ala	Tyr	Gln	Val	Arg
196	Asn	Ser	Thr	Gly	Leu	Tyr	His	Val	Thr	Asn	Asp	Cys	Pro	Asn	Ser
211	Ser	Ile	Val	Tyr	Glu	Ala	His	Asp	Ala	Ile	Leu	His	Thr	Pro	Gly
226	Cys	Val	Pro	Cys	Val	Arg	Glu	Gly	Asn	Val	Ser	Arg	Cys	Trp	Val
241	Ala	Met	Thr	Pro	Thr	Val	Ala	Thr	Arg	Asp	Gly	Lys	Leu	Pro	Ala
256	Thr	Gln	Leu	Arg	Arg	His	Ile	Asp	Leu	Leu	Val	Gly	Ser	Ala	Thr
271	Leu	Cys	Ser	Ala	Leu	Tyr	Val	Gly	Asp	Leu	Cys	Gly	Ser	Val	Phe
286	Leu	Ile	Gly	Gln	Leu	Phe	Thr	Phe	Ser	Pro	Arg	Arg	His	Trp	Thr
301	Thr	Gln	Gly	Cys	Asn	Cys	Ser	Ile	Tyr	Pro	Gly	His	Ile	Thr	Gly
316	His	Arg	Met	Ala	Trp	Asp	Met	Met	Met	Asn	Trp	Ser	Pro	Thr	Ala
331	Ala	Leu	Val	Met	Ala	Gln	Leu	Leu	Arg	Ile	Pro	Gln	Ala	Ile	Leu
346	Asp	Met	Ile	Ala	Gly	Ala	His	Trp	Gly	Val	Leu	Ala	Gly	Ile	Ala
361	Tyr	Phe	Ser	Met	Val	Gly	Asn	Trp	Ala	Lys	Val	Leu	Val	Val	Leu
376	Leu	Leu	Phe	Ala	Gly	Val	Asp	Ala	Glu	Thr	Ile	Val	Ser	Gly	Gly
391	Gln	Ala	Ala	Arg	Ala	Met	Ser	Gly	Leu	Val	Ser	Leu	Phe	Thr	Pro
406	Gly	Ala	Lys	Gln	Asn	Ile	Gln	Leu	Ile	Asn	Thr	Asn	Gly	Ser	Trp
421	His	Ile	Asn	Ser	Thr	Ala	Leu	Asn	Cys	Asn	Glu	Ser	Leu	Asn	Thr
436	Gly	Trp	Leu	Ala	Gly	Leu	Ile	Tyr	Gln	His	Lys	Phe	Asn	Ser	Ser
451	Gly	Cys	Pro	Glu	Arg	Leu	Ala	Ser	Cys	Arg	Pro	Leu	Thr	Asp	Phe
466	Asp	Gln	Gly	Trp	Gly	Pro	Ile	Ser	Tyr	Ala	Asn	Gly	Ser	Gly	Pro
481	Asp	Gln	Arg	Pro	Tyr	Cys	Trp	His	Tyr	Pro	Pro	Lys	Pro	Cys	Gly
496	Ile	Val	Pro	Ala	Lys	Ser	Val	Cys	Gly	Pro	Val	Tyr	Cys	Phe	Thr
511	Pro	Ser	Pro	Val	Val	Val	Gly	Thr	Thr	Asp	Arg	Ser	Gly	Ala	Pro
526	Thr	Tyr	Ser	Trp	Gly	Glu	Asn	Asp	Thr	Asp	Val	Phe	Val	Leu	Asn
541	Asn	Thr	Arg	Pro	Pro	Leu	Gly	Asn	Trp	Phe	Gly	Cys	Thr	Trp	Met
556	Asn	Ser	Thr	Gly	Phe	Thr	Lys	Val	Cys	Gly	Ala	Pro	Pro	Cys	Val
571	Ile	Gly	Gly	Ala	Gly	Asn	Asn	Thr	Leu	His	Cys	Pro	Thr	Asp	Cys
586	Phe	Arg	Lys	His	Pro	Asp	Ala	Thr	Tyr	Ser	Arg	Cys	Gly	Ser	Gly
601	Pro	Trp	Ile	Thr	Pro	Arg	Cys	Leu	Val	Asp	Tyr	Pro	Tyr	Arg	Leu
616	Trp	His	Tyr	Pro	Cys	Thr	Ile	Asn	Tyr	Thr	Ile	Phe	Lys	Ile	Arg
631	Met	Tyr	Val	Gly	Gly	Val	Glu	His	Arg	Leu	Glu	Ala	Ala	Cys	Asn
646	Trp	Thr	Arg	Gly	Glu	Arg	Cys	Asp	Leu	Glu	Asp	Arg	Asp	Arg	Ser
661	Glu	Leu	Ser	Pro	Leu	Leu	Leu	Thr	Thr	Thr	Gln	Trp	Gln	Val	Leu
676	Pro	Cys	Ser	Phe	Thr	Thr	Leu	Pro	Ala	Leu	Ser	Thr	Gly	Leu	Ile
691	His	Leu	His	Gln	Asn	Ile	Val	Asp	Val	Gln	Tyr	Leu	Tyr	Gly	Val

Figure 1. Continued.

706 Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Val Val
721 Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu
736 Trp Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn
751 Leu Val Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly Leu
766 Val Ser Phe Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly
781 Lys Trp Val Pro Gly Ala Val Tyr Thr Phe Tyr Gly Met Trp Pro
796 Leu Leu Leu Leu Leu Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu
811 Asp Thr Glu Val Ala Ala Ser Cys Gly Gly Val Val Leu Val Gly
826 Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys Arg Tyr Ile Ser
841 Trp Cys Leu Trp Trp Leu Gln Tyr Phe Leu Thr Arg Val Glu Ala
856 Gln Leu His Val Trp Ile Pro Pro Leu Asn Val Arg Gly Gly Arg
871 Asp Ala Val Ile Leu Leu Met Cys Ala Val His Pro Thr Leu Val
886 Phe Asp Ile Thr Lys Leu Leu Leu Ala Val Phe Gly Pro Leu Trp
901 Ile Leu Asp Ala Ser Leu Leu Lys Val Pro Tyr Phe Val Arg Val
916 Gln Gly Leu Leu Arg Phe Cys Ala Leu Ala Arg Lys Met Ile Gly
931 Gly His Tyr Val Gln Met Val Ile Ile Lys Leu Gly Ala Leu Thr
946 Gly Thr Tyr Val Tyr Asn His Leu Thr Pro Leu Arg Asp Trp Ala
961 His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val
976 Phe Ser Gln Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr
991 Ala Ala Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg
1006 Arg Gly Arg Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser
1021 Lys Gly Trp Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln
1036 Thr Arg Gly Leu Leu Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg
1051 Asp Lys Asn Gln Val Glu Gly Glu Val Gln Ile Val Ser Thr Ala
1066 Ala Gln Thr Phe Leu Ala Thr Cys Ile Asn Gly Val Cys Trp Thr
1081 Val Tyr His Gly Ala Gly Thr Arg Thr Ile Ala Ser Pro Lys Gly
1096 Pro Val Ile Gln Met Tyr Thr Asn Val Asp Gln Asp Leu Val Gly
1111 Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu Thr Pro Cys Thr Cys
1126 Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala Asp Val Ile
1141 Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser Pro
1156 Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu Leu
1171 Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
1186 Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn
1201 Leu Glu Thr Thr Met Arg Ser Pro Val Phe Trp Asp Asn Ser Ser
1216 Pro Pro Val Val Pro Gln Ser Phe Gln Val Ala His Leu His Ala
1231 Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala
1246 Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala
1261 Thr Leu Gly Phe Gly Ala Tyr Met Ser Lys Ala His Gly Ile Asp
1276 Pro Asn Ile Arg Thr Gly Val Arg Thr Ile Thr Thr Gly Ser Pro
1291 Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys
1306 Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser
1321 Thr Asp Ala Thr Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln
1336 Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr Ala Thr
1351 Pro Pro Gly Ser Val Thr Val Pro His Pro Asn Ile Glu Glu Val
1366 Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile
1381 Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile Phe Cys His
1396 Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala Leu
1411 Gly Ile Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
1426 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu
1441 Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn
1456 Thr Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe
1471 Thr Ile Glu Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr
1486 Gln Arg Arg Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg
1501 Phe Val Ala Pro Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser
1516 Val Leu Cys Glu Cys Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu

Figure 1. Continued.

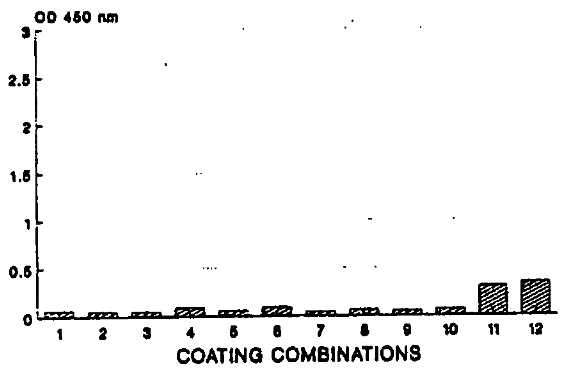
1531 Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Met Asn Thr
1546 Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Gly
1561 Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe Leu Ser Gln
1576 Thr Lys Gly Ser Gly Glu Asn Leu Pro Tyr Leu Val Ala Tyr Gln
1591 Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp Asp
1606 Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly
1621 Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Ile
1636 Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met Ser
1651 Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly
1666 Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val
1681 Val Ile Val Gly Arg Val Val Leu Ser Gly Lys Pro Ala Ile Ile
1696 Pro Asp Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu
1711 Cys Ser Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala
1726 Glu Gln Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser
1741 Arg Gln Ala Glu Val Ile Ala Pro Ala Val Gln Thr Asn Trp Gln
1756 Lys Leu Glu Thr Phe Trp Ala Lys His Met Trp Asn Phe Ile Ser
1771 Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro
1786 Ala Ile Ala Ser Leu Met Ala Phe Thr Ala Ala Val Thr Ser Pro
1801 Leu Thr Thr Ser Gln Thr Leu Leu Phe Asn Ile Leu Gly Gly Trp
1816 Val Ala Ala Gln Leu Ala Ala Pro Gly Ala Ala Thr Ala Phe Val
1831 Gly Ala Gly Leu Ala Gly Ala Ala Ile Gly Ser Val Gly Leu Gly
1846 Lys Val Leu Ile Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val Ala
1861 Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu Val Pro Ser
1876 Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro Gly
1891 Ala Leu Val Val Gly Val Val Cys Ala Ala Ile Leu Arg Arg His
1906 Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile
1921 Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val
1936 Pro Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser
1951 Leu Thr Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser
1966 Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile
1981 Trp Asp Trp Ile Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu
1996 Lys Ala Lys Leu Met Pro Gln Leu Pro Gly Ile Pro Phe Val Ser
2011 Cys Gln Arg Gly Tyr Lys Gly Val Trp Arg Val Asp Gly Ile Met
2026 His Thr Arg Cys His Cys Gly Ala Glu Ile Thr Gly His Val Lys
2041 Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr Cys Arg Asn Met
2056 Trp Ser Gly Thr Phe Pro Ile Asn Ala Tyr Thr Thr Gly Pro Cys
2071 Thr Arg Leu Pro Ala Pro Asn Tyr Thr Phe Ala Leu Trp Arg Val
2086 Ser Ala Glu Glu Tyr Val Glu Ile Arg Gln Val Gly Asp Phe His
2101 Tyr Val Thr Gly Met Thr Thr Asp Asn Leu Lys Cys Pro Cys Gln
2116 Val Pro Ser Pro Glu Phe Phe Thr Glu Leu Asp Gly Val Arg Leu
2131 His Arg Phe Ala Pro Pro Cys Lys Pro Leu Leu Arg Glu Glu Val
2146 Ser Phe Arg Val Gly Leu His Glu Tyr Pro Val Gly Ser Gln Leu
2161 Pro Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu
2176 Thr Asp Pro Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu
2191 Ala Arg Gly Ser Pro Pro Ser Val Ala Ser Ser Ser Ala Ser Gln
2206 Leu Ser Ala Pro Ser Leu Lys Ala Thr Cys Thr Ala Asn His Asp
2221 Ser Pro Asp Ala Glu Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln
2236 Glu Met Gly Gly Asn Ile Thr Arg Val Glu Ser Glu Asn Lys Val
2251 Val Ile Leu Asp Ser Phe Asp Pro Leu Val Ala Glu Glu Asp Glu
2266 Arg Glu Ile Ser Val Pro Ala Glu Ile Leu Arg Lys Ser Arg Arg
2281 Phe Ala Gln Ala Leu Pro Val Trp Ala Arg Pro Asp Tyr Asn Pro
2296 Pro Leu Val Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro Pro Val
2311 Val His Gly Cys Pro Leu Pro Pro Pro Lys Ser Pro Pro Val Pro
2326 Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr Glu Ser Thr Leu
2341 Ser Thr Ala Leu Ala Glu Leu Ala Thr Arg Ser Phe Gly Ser Ser

Figure 1. Continued.

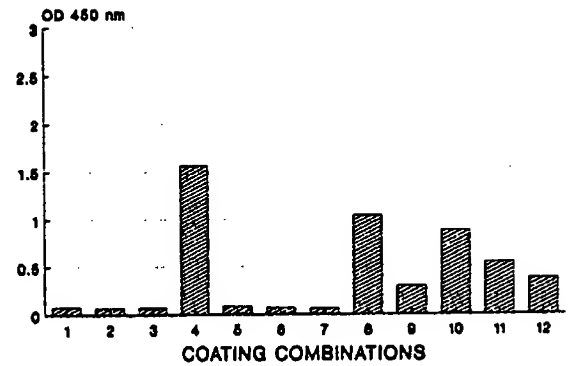
2356 Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser Ser Glu
2371 Pro Ala Pro Ser Gly Cys Pro Pro Asp Ser Asp Ala Glu Ser Tyr
2386 Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu
2401 Ser Asp Gly Ser Trp Ser Thr Val Ser Ser Glu Ala Asn Ala Glu
2416 Asp Val Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Cys
2431 Val Thr Pro Cys Ala Ala Glu Glu Gln Lys Leu Pro Ile Asn Ala
2446 Leu Ser Asn Ser Leu Leu Arg His His Asn Leu Val Tyr Ser Thr
2461 Thr Ser Arg Ser Ala Cys Gln Arg Gln Lys Lys Val Thr Phe Asp
2476 Arg Leu Gln Val Leu Asp Ser His Tyr Gln Asp Val Leu Lys Glu
2491 Val Lys Ala Ala Ala Ser Lys Val Lys Ala Asn Leu Leu Ser Val
2506 Glu Glu Ala Cys Ser Leu Thr Pro Pro His Ser Ala Lys Ser Lys
2521 Phe Gly Tyr Gly Ala Lys Asp Val Arg Cys His Ala Arg Lys Ala
2536 Val Thr His Ile Asn Ser Val Trp Lys Asp Leu Leu Glu Asp Asn
2551 Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu Val Phe
2566 Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu Ile
2581 Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu
2596 Tyr Asp Val Val Thr Lys Leu Pro Leu Ala Val Met Gly Ser Ser
2611 Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val
2626 Gln Ala Trp Lys Ser Lys Lys Thr Pro Met Gly Phe Ser Tyr Asp
2641 Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr
2656 Glu Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg
2671 Val Ala Ile Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro
2686 Leu Thr Asn Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys Arg
2701 Ala Ser Gly Val Leu Thr Thr Ser Cys Gly Asn Thr Leu Thr Cys
2716 Tyr Ile Lys Ala Arg Ala Ala Cys Arg Ala Ala Gly Leu Gln Asp
2731 Cys Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Cys Glu
2746 Ser Ala Gly Val Gln Glu Asp Ala Ala Ser Leu Arg Ala Phe Thr
2761 Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp Pro Pro Gln
2776 Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser Asn Val
2791 Ser Val Ala His Asp Gly Ala Gly Lys Arg Val Tyr Tyr Leu Thr
2806 Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala
2821 Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe
2836 Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe
2851 Ser Val Leu Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asp Cys
2866 Glu Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro
2881 Pro Ile Ile Gln Arg Leu Gly Cys Pro Glu Arg Leu Ala Ser

Figure 2. Antibody binding to individual peptides and various mixtures in an ELISA assay.

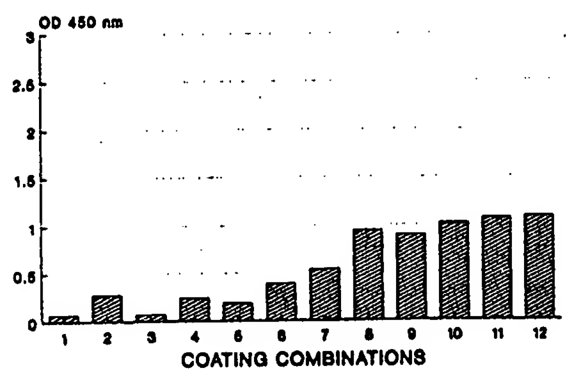
PEPTIDE COATING COMBINATIONS
SAMPLE 262



PEPTIDE COATING COMBINATIONS
SAMPLE 284 (undiluted)



PEPTIDE COATING COMBINATIONS
SAMPLE 287

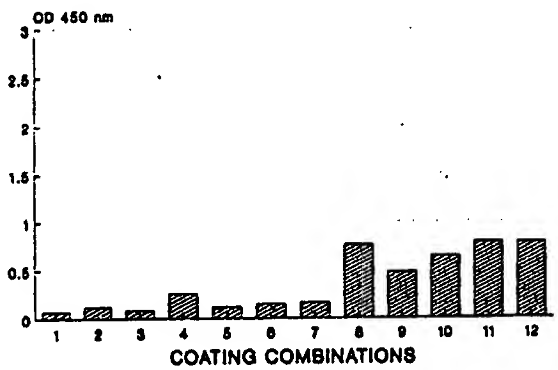


COATING COMBINATIONS USED

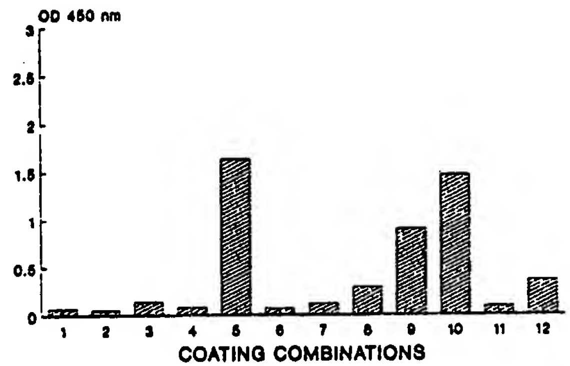
1: IX
2: XVIII
3: I
4: III
5: V
6: IX+XVIII
7: I+XVIII
8: I+III+IX
9: I+III+V+XVIII
10: I+III+V+IX
11: I+III+IX+XVIII
12: I+III+V+IX+XVIII

Figure 2. continued.

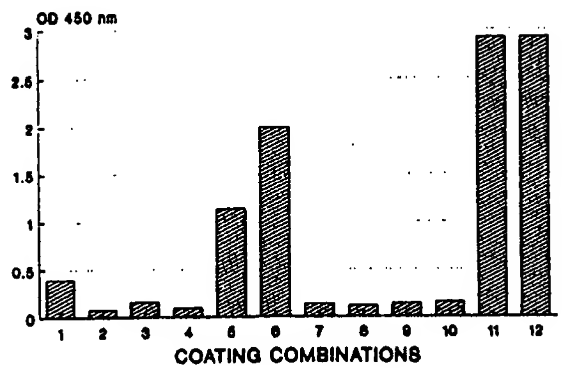
PEPTIDE COATING COMBINATIONS
SAMPLE 266



PEPTIDE COATING COMBINATIONS
SAMPLE 272



PEPTIDE COATING COMBINATIONS
SAMPLE 273

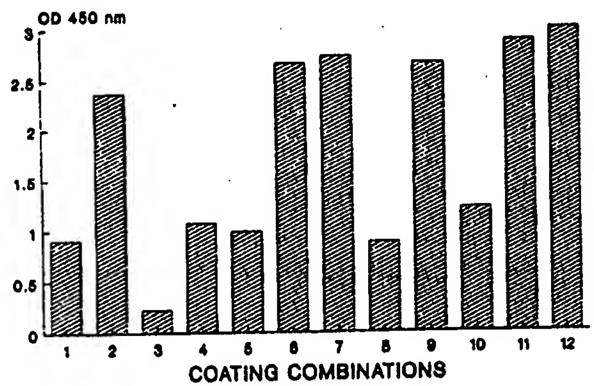


COATING COMBINATIONS USED

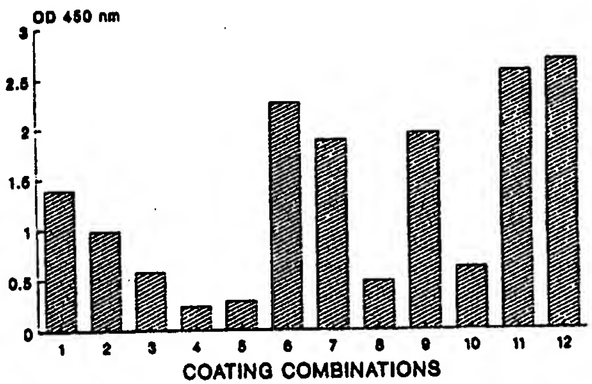
1: IX
2: XVIII
3: I
4: III
5: V
6: IX+XVIII
7: I+XVIII
8: I+III+IX
9: I+III+V+XVIII
10: I+III+V+IX
11: I+III+IX+XVIII
12: I+III+V+IX+XVIII

Figure 2. continued.

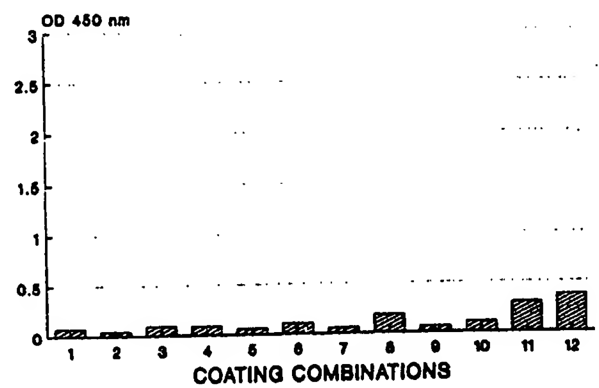
PEPTIDE COATING COMBINATIONS
SAMPLE 278



PEPTIDE COATING COMBINATIONS
SAMPLE 282



PEPTIDE COATING COMBINATIONS
SAMPLE 8247

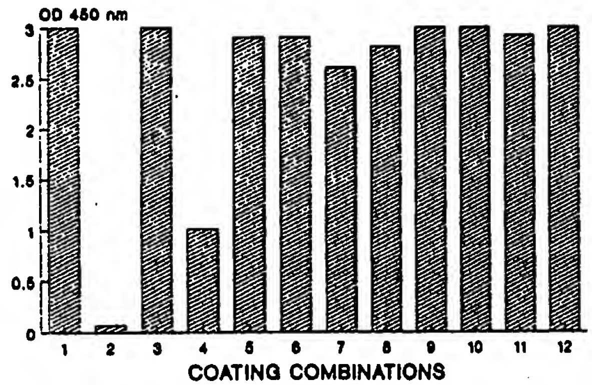


COATING COMBINATIONS USED

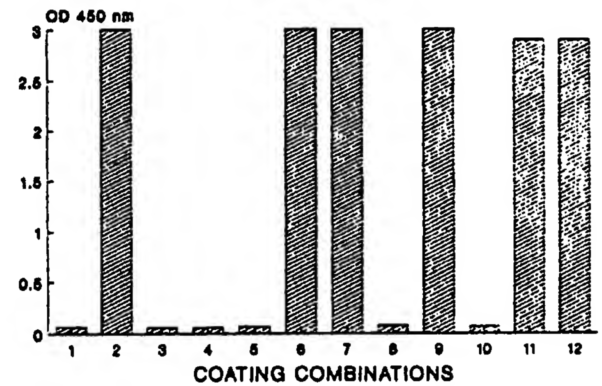
1: IX
2: XVIII
3: I
4: III
5: V
6: IX+XVIII
7: I+XVIII
8: I+III+IX
9: I+III+V+XVIII
10: I+III+V+IX
11: I+III+IX+XVIII
12: I+III+V+IX+XVIII

Figure 2. continued.

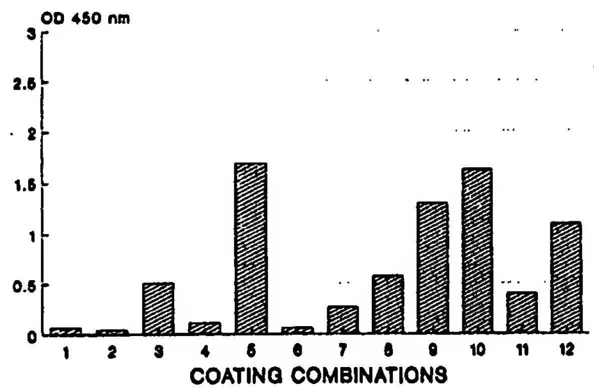
PEPTIDE COATING COMBINATIONS
SAMPLE 8287



PEPTIDE COATING COMBINATIONS
SAMPLE 8290



PEPTIDE COATING COMBINATIONS
SAMPLE 257



COATING COMBINATIONS USED

1: IX
2: XVIII
3: I
4: III
5: V
6: IX+XVIII
7: I+XVIII
8: I+III+IX
9: I+III+V+XVIII
10: I+III+V+IX
11: I+III+IX+XVIII
12: I+III+V+IX+XVIII

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 12 4241

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	EP-A-0 388 232 (CHIRON CORP.) * Entire document, especially page 4, line 58 - page 5, line 9; page 5, lines 23-29; page 6, lines 17-56; claims 10-12,18,21-23; figure 17 * -----	1-7,20-25	C 07 K 7/10 G 01 N 33/576 A 61 K 39/29
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 K A 61 K G 01 N

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	16-08-1991	GROENENDIJK M.S.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

European Patent
Office

EP 90 12 4241

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,
namely:

See sheet -B-

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims,

namely claims: 1-7 and 20-25(partially)

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims 1-7 and 20-25(partially): Peptides of the formula I-VII, their compositions and use as diagnostic.
2. Claims 8-14 and 20-25(partially): Peptides of the formula VIII-XIV, their compositions and use as diagnostic.
3. Claims 15-19 and 20-25(partially): Peptides of the formula XV-XIX, their compositions and use as diagnostic.